Recombinant Human Heparan Sulfate 6-O-Sulfotransferase 3/HS6ST3

Catalog Number: 8568-ST

DESCRIPTION

Source	Chinese Hamster Ovary cell line, CHO-derived Pro28-Trp471, with an N-terminal 6-His tag Accession # Q8IZP7
N-terminal Sequence Analysis	His
Predicted Molecular Mass	52 kDa

SPECIFICATIONS

SDS-PAGE	60-95 kDa, reducing conditions
Activity	Measured by its ability to transfer sulfate from PAPS to heparan sulfate. The specific activity is >65 pmol/min/µg, as measured under the described conditions.
Endotoxin Level	<1.0 EU per 1 µg of the protein by the LAL method.
Purity	>95%, by SDS-PAGE under reducing conditions and visualized by Colloidal Coomassie® Blue stain at 5 µg per lane.
Formulation	Supplied as a 0.2 µm filtered solution in Tris and NaCl. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Universal Sulfotransferase Activity Kit (Catalog # EA003)
- 10X Assay Buffer (supplied in kit): 500 mM Tris, 150 mM $MgCl_2$, pH 7.5
- Recombinant Human Heparan Sulfate 6-O-Sulfotransferase 3/HS6ST3 (rhHS6ST3) (Catalog # 8568-ST)
- Donor Substrate: $PAP^{32}S$ (3'-Phosphoadenosine-5'-Phosphosulfate) (Catalog # ES019)
- Acceptor Substrate: Heparan Sulfate (Celsus Labs, Catalog # HO-3105), 50 mg/mL stock in deionized water
- 96-well Clear Plate (Catalog # DY990)
- Plate Reader (Model: SpectraMax Plus by Molecular Devices) or equivalent

Assay

1. Prepare 1X Assay Buffer by combining 10X stock and diluting 10 fold with deionized water.
2. Dilute 1 mM Phosphate Standard by adding 40 µL of the 1 mM Phosphate Standard to 360 µL of 1X Assay Buffer for a 100 µM stock. This is the first point of the standard curve.
3. Continue standard curve by performing six one-half serial dilutions of the 100 µM Phosphate stock in 1X Assay Buffer. The standard curve has a range of 0.078 to 5 nmol per well.
4. Load 50 µL of each dilution of the standard curve into a plate. Include a curve blank containing 50 µL of 1X Assay Buffer.
5. Prepare a reaction mixture containing 0.4 mM $PAP^{32}S$ and 6 mg/mL Heparin Sulfate in 1X Assay Buffer.
6. Dilute Coupling Phosphatase 3 (supplied in kit) to 50 µg/mL in 1X Assay Buffer.
7. Dilute rhHS6ST3 to 33.34 µg/mL in 1X Assay Buffer.
8. Load 15 µL of the 33.34 µg/mL rhHS6ST3 into empty wells of the same plate as the curve. Include a control containing 15 µL of 1X Assay Buffer.
9. Add 10 µL of 50 µg/mL Coupling Phosphatase 3 to wells containing enzyme and control, excluding the standard curve.
10. Add 25 µL of reaction mixture to the wells, excluding the standard curve.
11. Seal plate and incubate at 37 ºC for 20 minutes.
12. Add 30 µL of the Malachite Green Reagent A to all wells.
13. Add 100 µL of deionized water to all wells. Mix briefly.
14. Add 30 µL of the Malachite Green Reagent B to all wells. Mix and incubate for 20 minutes at room temperature.
15. Read plate at 620 nm (absorbance) in endpoint mode.
16. Calculate specific activity:

$$\text{Specific Activity (pmol/min/µg)} = \frac{\text{Phosphate released* (nmol) x (1000 pmol/nmol)}}{\text{Incubation time (min) x amount of enzyme (µg)}}$$

*Derived from the phosphate standard curve using linear or 4-parameter fitting and adjusted for Control.

Final Assay Conditions

Per Reaction:

- rhHS6ST3: 0.5 µg
- Coupling Phosphatase 3: 0.5 µg
- Heparan Sulfate: 150 µg
- $PAP^{32}S$: 0.2 mM

PREPARATION AND STORAGE

Shipping	The product is shipped with polar packs. Upon receipt, store it immediately at the temperature recommended below.
Stability & Storage	**Use a manual defrost freezer and avoid repeated freeze-thaw cycles.** • 6 months from date of receipt, -20 to -70 °C as supplied. • 3 months, -20 to -70 °C under sterile conditions after opening.

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BACKGROUND

Heparan sulfate is a highly sulfated polysaccharide that can be found on the cell surface and extracellular matrix. It is usually covalently attached to a protein core as the glycan component of a proteoglycan. Heparan sulfate interacts with a variety of proteins, such as growth factors, protease inhibitors, cytokines, lipoprotein lipase and viral envelope proteins, therefore playing roles in cell growth, cell differentiation, cell motility, blood coagulation, lipid metabolism and viral infection (1, 2). Heparan sulfate consists of repeating residues of uronic acid and Nacetylglucosamine. The uronic acid residues can be sulfated at the 2-O position by heparan sulfate 2-O-sulfotransferase. The N-acetylglucosamine residues can be sulfated at N-, 3-O, and 6-O positions by N-deacetylase/N-sulfotransferases, heparan sulfate 3-O and 6-O sulfotransferases, respectively. However, the reactions catalyzed by these sulfotransferases are normally incomplete on the whole chain of heparan sulfate. As a result, heparan sulfate displays enormous sequence diversity that allows it to interact with a wide spectrum of proteins differently. Three heparan sulfate 6-O-sulfotransferases are found both in human and mouse possibly with overlapping substrate specificity (3). HS6ST3 is ubiquitously expressed while HS6ST1 and HS6ST2 are expressed primarily in the liver and brain/spleen, respectively. The enzyme activity of the recombinant HS6ST3 is measured using a phosphatase-coupled assay (7).

References:

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